Pyrethroid use-malaria control and individual applications by households for other pests and home garden use.

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Abstract

Presence of pyrethroid insecticides in human breast milk and in thatch wall material of dwellings from Southern Africa subtropical area (Manhiça, Mozambique) was investigated to assess potential pyrethroid route of human exposure. Human breast milk samples were collected during 2002 when pyrethroids were widely used as insecticides for mosquito bed nets in Mozambique for malaria control. The median concentration value of total pyrethroids ranged between 87 and 1200 ng/g lw, with λ-cyhalothrin being the most predominant pyrethroid in human breast milk contributing for 35% of the total amount. Moreover, and for the first time, an isomer-specific enrichment was found in human breast milk, showing a selective isomeric accumulation or metabolism in the human body.

Based on the calculated pyrethroid concentrations in human breast milk, the daily ingestion rate of pyrethroid was estimated. The nursing infant dietary intake ranged from 0.67 to 9.0 µg (kg of body weight)$^{-1}$ day^{-1}. In addition, thatch materials collected after the reintegration of dichlorodiphenyl-trichloroethene (DDT) as insecticide residual spraying (IRS) in Mozambique, showed the presence of pyrethroids with concentration values ranging between 6.9 and 700 ng/g dw. In thatch material as well as in human breast milk, pyrethroid contamination was mainly attributed to the agriculture usage of this insecticide knowing that agriculture represent the 80% of the economy in Mozambique. However, a possible usage of this insecticide as IRS in Mozambique cannot be excluded despite their low efficiency for malaria control. The continued use of these compounds (both for agricultural and malaria prevention) and the ingestion rates calculated from the breast milk concentrations indicate that these insecticides cannot be overlooked for the assessment of the lactation risks of breastfeeding infants from the Manhiça region.

Introduction

In 2004, an estimated 350-500 million people contracted malaria and 0.85 million died (91% in Africa, 85% of them children under 5 years) (WHO 2007a, WHO and UNICEF 2005). The World Health Organization Pesticide Evaluation Scheme (WHOPES) (WHO 2008) supports the use of recommended insecticides for malaria control based on the evaluation of human and environmental safety conditions (WHO 2006). In tropical Africa, these insecticides were used for treatment of mosquito nets (ITNs) (Kapp, 2004) and indoor residual spraying (IRS) on walls and roofs to kill the mosquitoes that land and rest there (Montgomery et al., 2010). In the last years increases of international funding for malaria control allowed to protecting larger numbers of people in sub-Saharan Africa, from 13 million in 2005 to 75 million in 2009 by IRS as well as 66% of the 765 million at risk by use of 254 million ITNs between 2008 and 2010 (World Malaria Report, WHO 2010). The implementation of dichlorodiphenyltrichloroethene (DDT) and pyrethroids for IRS

52 constitute one of the major interventions for reduction and interruption of malaria transmission by
53 vector control in all epidemiological settings (World Malaria Report, WHO 2010).

54 In Mozambique DDT was introduced in 1946 for agriculture and health programs. The IRS
55 program with DDT broke down in the late 1970s due to the civil war. After this event (1993), the
56 National Malaria Control Program (MNMCP) decided to restart IRS with pyrethroids in suburban
57 areas of most provincial capitals. However, *Anopheles funestus*, one of the main mosquito vectors
58 became resistant to this group of insecticides (Hargreaves et al., 2000; Sereda and Meinhardt,
59 2005). Thus, in 2000 carbamates (bendiocarb) were used in the rural areas of Maputo province
60 within a coordinated effort for protection of the population of the Lubombo region (Mozambique,
61 Swaziland and South Africa; Mabaso et al., 2004) while the use of pyrethroids was continued for
62 mosquito nets. By end 2005, DDT was reintroduced for IRS following the WHO recommendations
63 for areas of potential human life loss as consequence of unstable malaria transmission and
64 epidemics (WHO, 2006),

65 Pyrethroids are synthesized derivates of pyrethrins, which are natural insecticides produced
66 by certain species of chrysanthemum (*Chrysanthemum cinerariaefolium*). Even though effects to
67 humans are still unclear, the US Environmental Protection Agency (EPA) has classified some of
68 them (cypermethrin, permethrin and biphenthrin) as possible human carcinogens (Cox, 1996).
69 Pyrethroids are persistent compounds with high hydrophobicity ($\log K_{ow}$ = 5.7-7.6) and low water
70 solubility (a few μg L^{-1}) (Laskowski, DA., 2002). Despite these properties there is evidence of
71 human pyrethroid metabolism and urine excretion of these compounds (ATSDR, 2003).

72 The accumulation of some pyrethroids in human milk has been considered in a limited
73 number of studies (Bouwman et al., 2006; Sereda et al., 2009; Zehringer and Herrmann, 2001)
74 showing appreciable pyrethroid levels in breast milk together with DDT. In some individuals,
75 pyrethroid levels were higher than DDT levels suggesting domestic and home garden use of the
76 former, while the presence of DDT was attributed to activities for control of malaria vectors. Except
77 for DDT, safety of insecticide residues in breast milk has not been considered during the WHOPES

evaluation and very little is known on the effect of these chemicals to infants. This issue is important because milk is the best sole nutrient source for infants, particularly in Africa.

In the present study assessment of pyrethroid exposure in a rural area located in the south of Mozambique (Manhiça district) is undertaken. The study encompasses a comprehensive examination of the compounds belonging to the pyrethroid group, e.g. bifenthrin, λ-cyhalothrin, permethrin, cyfluthrin, cypermethrin, esfenvalerate, fenvalerate, fenpropathrin, deltamethrin, tetramethrin, phenothrin and resmethrin, including the isomeric composition of some of these compounds. Human milk was analysed as body burden estimate. Moreover, pyrethroid content in walls (thatch material) of dwellings was also determined for assessment of potential human exposure. To the best of our knowledge this is the first time in which this combined human-environmental approach is addressed.

2. Material and Method

2.1. Study area

Manhiça district is a rural area located in the Northern of Maputo province in Mozambique. The climate is subtropical characterized by a warm and rainy season between November-April and a dry and cold season during the rest of the year.

2.2. Samples

Mature breast milk samples were collected in 2002 (n = 22) in the context of studies conducted at the Centro de Investigação em Saúde da Manhiça (CISM). The research protocol was approved by the ethic committees of Mozambique and Hospital Clinic in Barcelona. All women signed an informed consent before they were enrolled in the study. Samples were stored in sterile polyester containers at -80ºC at CISM and at -20°C in IDÆA-CSIC until analysis, which was performed in this institute.

Thatch samples (n = 14) covering surfaces of about 10 cm^2 were collected in-door during 2006-2007 and introduced in sterile polyester bags (Kapak Corporation, Minneapolis, USA) which were closed with a heat sealer and stored at -20°C.

Thatch was elaborated from *Typha* plants (particulary *Typha latifolia*).

2.3. Standards and reagents

All certified pyrethroid standards were obtained from Dr. Ehrenstorfer (Augsburg, Germany). They encompassed a standard mixture of seven pyrethroids, cyfluthrin, cypermethrin, deltamethrin, fenvalerate, permethrin, phenothrin and tetramethrin and single analytical standards of bifenthrin, λ-cyhalothrin, esfenvalerate, fenpropathrin and resmethrin. d_6-trans-permethrin and d_6-trans-cypermethrin were used as surrogate standard. Hexane, dichloromethane and acetonitrile were obtained from Sigma Aldrich (St. Louis, MO, USA). The solvents used in this study were all pesticide grade.

The standard solutions were prepared in ethyl acetate. In order to check the linearity of the method two calibration curves were prepared at five different concentrations ranging between 0.08 and 2.5 ng mL^{-1} (first curve) and between 5 and 45 ng mL^{-1} (second curve). These calibration lines contained d_6-trans-permethryn and d_6-trans-cypermethrin at 45 ng $mL^{-1}$and 22 ng mL^{-1}, respectively.

2.4. Sample preparation

Thatch material (0.3 g) and breast milk (0.1 g dry weight) were placed in 40 mL glass-centrifuge tubes. They were fortified with d_6-trans-permethrin (4.5 ng) and d_6-trans-cypermethrin (2.5 ng) as surrogate standards. The samples were stirred and extracted by sonication with 20 ml of hexane:dichloromethane (2:1) in a Raypa, UCI-200 bath for 15 min. Then, the samples were centrifuged at 3500 rpm for 5 min. The organic phase remained at the top of the conical tube and

was entirely transferred to a vial and evaporated under a nitrogen stream. This extraction step was repeated two additional times and all the solvent residues were collected together.

Thatch material extracts were cleaned up by elution through Florisil cartridges (2g/15 ml). Each cartridge was conditioned with 15 mL of ethyl acetate:dichloromethane (2:1). The sample was loaded onto the cartridges and the pyrethroids were eluted with 25 mL of ethyl acetate. The eluate was evaporated under a nitrogen stream and re-dissolved with 100 µL ethyl acetate for GC-NCI-MS-MS analysis (Feo et al., 2010).

The breast milk extracts were cleaned up by elution through C18 cartridges (2g/15ml) coupled to basic alumina (5g/ 25ml) and conditioned with 25 ml of acetonitrile. Then the sample was eluted with 30 ml of acetonitrile. The acetonitrile extract was evaporated under a nitrogen stream and the residue was dissolved in 100 µl of ethyl acetate for GC-NCI-MS-MS analysis.

2.5. GC-NCI-MS-MS operating conditions

GC-MS-MS analysis was performed in NCI mode on Agilent Technologies 7890A GC system coupled to 7000A GC/MS Triple Quad. A DB-5MS capillary column (15m x 0.25mm i.d., 0.1 µm film thickness) containing 5% phenyl methyl siloxane was used with helium as carrier gas at constant flow of 1 ml min^{-1}. The temperature program was from 100°C (held for 1 min) to 230°C at 15°C min^{-1}, then from 230 to 310°C (held for 2 min) at 10°C min^{-1}, using the splitless injection mode during 0.8 min. Inject volume was 3 µl. The inlet temperature was set at 275°C and ion source temperature at 250°C. Ammonia was used as reagent gas at 2.04 x 10^{-4} torr. More details on MS-MS condition and selected transitions were reported elsewhere (Feo et al., 2011).

2.6. Lipid content

Total milk lipid content was determined by crematocrit method (Mayans et al., 1994). However, due to the low breast milk volume available, lipid content was not calculated in all the collected samples, thus a median value was used for the calculation of pyrethroid concentrations.

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157 *2.7. Quality control*

158 Recovery tests were carried out by addition of each pyrethroid to a thatch sample at concentrations
159 of 16 ng/g dry weight (dw) and to a breast milk sample at concentrations of 100 ng/g lipid weight
160 (lw) (Table 1). These samples were previously analyzed in order to determine pyrethroid presence
161 before spiking. Five replicates were prepared for evaluation of the reproducibility of the method.
162 Recovery values were higher than 77% in straw and ranged between 48 and 91% in breast milk
163 with relative standard deviation values lower than 3-20% (n=5; Table 1). Method detection limits
164 **(**MLODs) defined as the minimum amount of analyte which produces a peak with a signal-to-noise
165 ratio equal to 3 were determined for each single pyrethroid isomers by estimating the relative
166 isomer abundance of the relative peak areas. They ranged between 0.10 to 75 pg/g dw and 3.1 to
167 1100 pg/g lw for straw and breast milk, respectively (Table 1). Limits of quantification, defined as
168 the minimum amount of analyte that produces a peak with a signal-to noise ratio equal to 10, ranged
169 between 0.33 to 230 pg/g and between 8.3 to 3600 pg/g lw for straw and breast milk, respectively.

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171 *2.8. Estimation of Nursing Infant Dietary Intake*

172 In order to evaluate the magnitude of exposure to pyrethroids by infants, the estimated daily intake
173 (EDI) was calculated as $EDI_i = C_i\ F\ Mb$ where EDI_i is the estimated intake [micrograms per
174 kilograms of body weight (bw) per day], C_i is the median concentration of pyrethroid in milk
175 samples (micrograms per grams of lipid weight), F is the lipid content in milk samples (grams of
176 lipid per 100 g of milk) and Mb is the daily consumption of milk (grams per kilograms of body
177 weight per day). The infant's average milk consumption (Mb), 175 g of milk $(kg\ bw)^{-1}$ day^{-1}, was
178 calculated from US EPA recommendations (US EPA, 2002) by assuming an average daily intake
179 breast milk rate of 702 mL of milk per day (723 g of milk per day) and a 1-month-old infant body
180 weight of 4.14 kg. The mean value of lipid content in analyzed samples was used for F estimation,
181 with a value of 4.4 g of lipid per 100 g of milk

3. Results and Discussion

3.1. Pyrethroid levels in human breast milk

Basic statistics of pyrethroid levels found in breast milk from Manhiça mothers are reported in Table 2. λ-Cyhalothrin and permethrin were detected in all samples followed by esfenvalerate/fenvalerate (found in 21 samples), cypermethrin (found in 20 samples) and tetramethrin and bifenthrin (found in 19 samples) while cyfluthrin was only detected in 9 samples. Phenothrin, resmethrin and deltamethrin were not found in any milk sample. The concentration ranges were 1.1-36, 16-440, 10-230, 11-220, 3.3-160, 9.7-200 and 6.7-230 pg/g lw for bifenthrin, λ-cyhalothrin, permethrin, cyfluthrin, cypermethrin, esfenvalerate/fenvalerate and tetramethrin, respectively. The median values estimated from case-wise data were 110 ng/g lw for λ-cyalothrin, 70 ng/g lw for tetramethrin, 60 ng/g lw for cyfluthrin, 55 ng/g lw for permethrin and 42 ng/g lw for esfenvalerate/fenvalerate. Total pyrethroid concentration ranged between 87 and 1200 ng/g lw. Figure 1 shows single pyrethroid contribution (%) to the total concentration. In this case, a pair-wise statistical approach was used: the most predominant pyrethroid was λ-cyhalothrin (35%) followed by permethrin (21%) cypermethrin, esfenvalerate/fenvalerate and tetramethrin (14%).

The literature on pyrethroid levels in human breast milk samples is very scarce. Our results can be compared to those found in human breast milk in Basle (Switzerland) during 1998/99 (Zehringer and Herrmann, 2001). In that study, 13 pyrethroids were analyzed with median concentration values ranging between 15 and 31 ng/g lw. In our study median concentration values of the 10 detected pyrethroid ranged between 87 and 1200 ng/g lw showing higher levels than those found in Basle. This was probably due to the different use of these insecticides. In Basle, pyrethroids were used only for agricultural and, in minor part, urban (e.g. pet sprays) applications (Zehringer and Herrmann, 2001).

More recently, Sereda et al., 2009 found high permethrin levels up to 1.2 µg/g lw (mean value) which occurred together with cypermethrin and cyfluthrin at lower concentration in human breast samples collected from northen KwaZulu Natal, South Africa (Sereda et al., 2009). The authors associated the pyrethroid contamination to home garden and indoor use. In the same region, during 2006 Bouwman et al. found permethrin, cyfluthrin, cypermethrin and delatmethrin at concentrations of 14.5, 42, 4.2 and 8.4 µg/l, respectively (∑ pyrethroid concentration of 31.5 µg/l) which the authors associated to agriculture (Bouwman et al., 2006). These levels are higher than those found in our study. According to the estimated fat content of 4% (Bouwmann et al., 2006) total pyrethroids ranged between 110 and 1050 with mean concentrations of 790 ng/g lw.

Pyrethroid concentrations found in Manhiça mothers are similar to those found in South Africa (Bouwman et al., 2006; Sereda et al., 2009).

3.2. Pyrethroid levels in thatch materials.

Basic statistics of pyrethroid levels found in thatch material are reported in Table 2. Cypermethrin was detected in 13 samples followed by λ-cyhalothrin and tetramethrin (detected in 12 samples). Cyfluthrin and esfenvalerate/fenvalerate were found in 11 samples. Permethrin was found in 9 samples while deltametrin, the pyrethroid used during the IRS program together with λ-cyhalothrin, were found in 8 samples. Bifenthrin, phenothrin and resmethrin were also found in a few samples. The concentration ranges were 0.45-7.7, 0.45-510, 0.45-695, 0.75-150, 0.50-210, 1.2-18, 2.9-30, 0.18-2.3, 0.52-3.1 and 0.05-0.76 ng/g dw for bifenthrin, λ-cyhalothrin, permethrin, cyfluthrin, cypermethrin, esfenvalerate/fenvalerate, deltamethrin, tetramethrin, phenothrin and resmethrin, respectively. The median values estimated from caisewise data were 7.4 ng/g dw for deltamethrin, 4.5 ng/g dw for cyfluthrin, 3.5 ng/g dw for λ-cyhalothrin, 3.2 ng/g dw for cypermethrin, 2.8 ng/g dw for permethrin and esfenvalerate/fenvalerate. Total pyrethroid concentration ranged between 7.0 and 700 ng/g dw. In Figure 1 the most predominant pyrethroid was cypermethrin (contribution of 37% of the total amount) followed by cyfluthrin (25%) and λ-cyhalothrin (19%).

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234 *3.3. Exposure and bioaccumulation of pyrethroids*

235 Pyrethroids are generally used in agriculture. This activity constitutes 80% of total income in
236 Mozambique, which traditionally was related to cotton and now also encompasses maize, soybeans
237 and rice (Arlindo and Keyser, 2007). In Manhiça people are mostly subsistence farmers and
238 workers in an agricultural cooperative that grows sugarcane, bananas and rice and also operate a
239 large sugarcane-processing factory (Alonso et al., 2002).

240 Pyrethroids have also been used for IRS being applied indoors and under the outside rafters
241 of dwellings. These insecticide compounds have also been used for ITN contributing to a reduction
242 of malaria transmission risk (Lindsay and Gibson 1998, Takken 2002). The use of λ-cyhalothrin and
243 deltamethrin for IRS was common (USAID, 2008) till the reintroduction of DDT in 2005 (WHO,
244 2006). In particular, deltametrin was used for malaria control since 1970, when it was an
245 impregnating agent in bed nets or curtains and later it was used for IRS in spite of its marked
246 excitorepellency, which in some situations may be an advantage as it reduces human-vector contact
247 (USAID, 2008). Deltamethrin was found in thatch samples collected during 2006-2007 but it was
248 not detected in any human breast milk sample collected in 2002. Conversely, λ-cyhalothrin was
249 found in both thatch and breast milk samples. The concentration of this compound was higher
250 (median value 110 ng/g lw) than that of the other pyrethroids identified.

251 The replacement period of thatch from the dwellings is generally four years. Taken into
252 account that thatch samples were collected indoor and that the half lives of pyrethroid range
253 between 11.5 and 425 days in aerobic and anaerobic soils and between 1.83 and 619 days in water
254 (Oros et al., 2005 and Laskowski et al., 2002), we can attribute the presence of pyrethroid
255 contamination in the dwelling thatch samples analyzed during 2006-2007 to agricultural
256 applications but also to their use for IRS.

257 Human exposure to pyrethroids, including λ-cyhalothrin, can result from various routes,
258 including dermal uptake, inhalation (dust and gas phase) and ingestion of food containing residues

of this group of insecticides (Bouwman and Kylin, 2009). The use of these compounds in agriculture and for IRS may therefore explain their occurrence in the milk samples collected in 2002.

Results on pyrethroid levels found in human breast milk demonstrate that mothers exposed to insecticide contamination accumulate pyrethroids that could be transfer to infants via breast milk. Some studies reported that pyrethroids are metabolized by humans: the chrysanthemic acid ester is usually cleaved via esterase or mixed function oxidase activity and the resulting alcohol moieties are converted to their corresponding acids. It is reported that these metabolites are partly conjugated to glucoronide and both the conjugates and free acids are excreted in urine (ATSDR, 2003). However, and based on our results, bioaccumulation of pyrethroid in women is evident and it seems to differ depending on the pyrethroid. Figure 1 shows the percentage contribution of each detected pyrethroid in thatch material and human breast milk. The distribution patterns are different which may indicate changes in pyrethroid use through time or the combination of different pyrethroid sources, e.g. domestic and agricultural applications, in breast milk.

Pyrethroid molecules typically contain 2-3 asymmetric carbon atoms (chiral centers), making them a family of pesticides with high chirality. Figure 2 shows the relative contributions of the two isomers of permethrin and esfenvalerate/fenvalerate found in a commercial technical mixture (standard), as well as in thatch material and human breast milk. For permethrin, the abundance in the commercial technical mixture is 84% and 16%, for isomer I and II respectively. In thatch material, an abundance of 69% and 31% was found, showing a roughly similar distribution. However, for human breast milk samples, the percentage contribution of both isomers is very similar, with 52% and 48%, respectively. The observed enrichment in isomer II may reflect higher bioaccumulation potential of this compound or, conversely, a higher degree of human metabolization of isomer I. To the best of our knowledge this selective enrichment in isomer composition is described here for the first time. Analysis of milk samples in forthcoming studies are needed for a better understanding of the processes leading to this preferential accumulation.

In the case of esfenvalerate/fenvalerate, the abundance in the commercial technical mixture is of 60% and 40%, for isomer I and II respectively. These percentages were similar to those found in thatch material and also in human breast milk samples, with 60% and 40% and 56% and 44% for isomer I and II in thatch material and human breast milk, respectively. In this case, no differential isomeric behavior was observed.

3.4. Nursing Infant Estimated Dietary Intake

EDI values calculated from the breastmilk concentrations (Table 2) were 0.12, 0.28, 1.5, 1.7, 1.75, 1.8 and 3.4 µg/kg bw and per day for cypermethrin, bifenthrin, esfenvalerate/fenvalerate, cyfluthrin, permethrin, tetramethrin and cyhalothrin, respectively (Table 3). These values were compared to the recommended acceptable daily intake (ADI) values reported by FAO and WHO 2005 for bifenthrin (4 µg (kg bw) $^{-1}$ day^{-1}, cyfluthrin (20 µg (kg bw) $^{-1}$ day^{-1}), cypermethrin (20 µg (kg bw) $^{-1}$ day^{-1}), deltamethrin (10 µg (kg bw) $^{-1}$ day^{-1}), λ-cyhalothrin (5 µg (kg bw) $^{-1}$ day^{-1}) and permethrin (50 µg (kg bw) $^{-1}$ day^{-1}) (FAO and WHO 2005 and FAO 2005). In general, the rates estimated from the mothers in Manhiça are lower than these recommended levels but the levels of λ-cyhalothrin in some mothers, the pyrethroid used during IRS program in Mozambique, were close to the ADI WHO-recommended value (5 µg (kg bw) $^{-1}$ day^{-1}).

Further comparison of these breast milk pyretroid concentrations can be obtained by comparison to the maximum residue levels (MRLs) after unit transformation in mg/kg of milk (FAO and WHO, 2005). These MRLs are proposed for food commodities or animal feeds. As shown in Table 4, the median concentrations of the six pyrethroids found in milk samples above limit of detection correspond to concentrations that are about six times lower than MRL values at the most. The mothers exhibiting highest concentrations have also values below these MRL reference levels.

4. Conclusions

Pyrethroids were found in human breast milk despite the discontinuation in the use of these compounds for IRS. Their occurrence may reflect an influence from the insecticide impregnated bed nets, agricultural sources or use for IRS in some cases despite their known low efficiency for malaria control. The presence of these compounds in breast milk confirms their bioaccumulation potential in humans. Some pyrethroid compounds are accumulated with isomeric discrimination. The concentrations of some of these insecticides found in some mothers, namely λ- cyhalothrin used for IRS in Mozambique, involves EDI values close to the upper limits recommended by FAO. The presence of pyrethroids in thatch from dwellings evidences that these insecticides are still used for IRS. The observed occurrence of pyrethroids in dwellings despite the preferential use of other insecticides for IRS and the concentrations of some of these compounds found in human breastmilk evidence that these compounds need to be considered in the evaluation of infant risks associated to lactation in areas where insecticides are used for elimination of malaria vectors.

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Figure Caption:

Figure 1. Percentage contribution of each pyrethroid to total contamination estimated in straw material and human breast milk collected from Manhiça (Mozambique).

Figure 2. Abundance (%) of the two isomers of permethrin and esfenvalerate/fenvalerate in straw material and human breast milk collected from Manhiça (Mozambique).

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Table 1. Analytical quality parameters of pyrethroid methodologies applied to straw material and breast milk samples.

	Straw material					Breast Milk				
	Blank (ng/g dw)	Recovery (%)	RSD (%)	MLODs[a] (pg/g dw)	MLOQs[a] (pg/g dw)	Blank (ng/g lw)	Recovery (%)	RSD (%)	MLODs[a] (pg/g lw)	MLOQs[a] (pg/g lw)
Bifenthrin	1.2	86	5	1.7	5.1	0.82	70	20	32	97
λ-Cyhalothrin	2.2	78	5	1.1	3.3	9.05	82	20	3.6	11
Permethrin	3.8	82	8	11; 9.5	33; 28.5	24	80	4	1100; 1100	3200; 3200
Cyfluthrin	1.1	85	20	2.7; 2.5; 0.10	8.1; 7.6; 0.30	nd	60	20	160; 160; 3.1	480; 480; 9.2
Cypermethrin	1.6	90	19	2.0; 4.5; 3.7	6.0; 13.5; 11	3.55	72	6	140; 140; 140	430; 430;430
Es/fenvalerate	2.8	98	20	1.0; 0.90	3.0; 2.7	nd	70	7	63; 63	190;190
Deltamethrin	3.0	104	20	13	39	nd	48	8	280	8.3
Tetramethrin	0.75	77	3	0.41; 0.75	1.2; 2.25	17	86	20	45	140
Phenothrin	0.84	60	20	67; 69	200; 210	nd	50	20	1100; 1100	3200; 3600
Resmethrin	Nd	87	20	69; 75	210; 230	nd	91	16	800; 780	2400; 2300
Total	17					54				

[a] MLODs and MLOQs were estimated for each isomer of a specific pyrethroid.

Table 2. Basic statistics for single pyrethroid and ∑ pyrethroid concentrations in straw materials (ng/g dw) and human breast milk (ng/g lw) of Mozambique.

	Bifenthrin	λ-Cyhalothrin	Permethrin	Cyfluthrin	Cypermethrin	Es/Fenvalerate	Deltamethrin	Tetramethrin	Phenothrin	Resmethrin	∑PYR
Straw											
Mean	2.9	59	80	17	24	5.4	11	0.68	1.4	0.43	162
Median	2.6	3.5	2.8	4.5	3.2	2.8	7.4	0.54	1.1	0.47	55
Max	7.7	510	695	150	210	18	30	2.3	3.1	0.76	700
Min	0.45	0.45	0.45	0.75	0.50	1.2	2.9	0.18	0.52	0.05	7.0
SD	2.6	150	230	45	60	5.2	9.1	0.59	1.0	0.36	220
Milk											
Mean	6.5	140	79	80	54	55	nd	80	nd	nd	425
Median	4.0	110	55	60	34	42	nd	70	nd	nd	370
Max	36	440	230	220	160	200	nd	230	nd	nd	1200
Min	1.1	16	10	11	3.3	9.7	nd	6.7	nd	nd	87
SD	8.0	120	62	65	50	44	nd	64	nd	nd	265

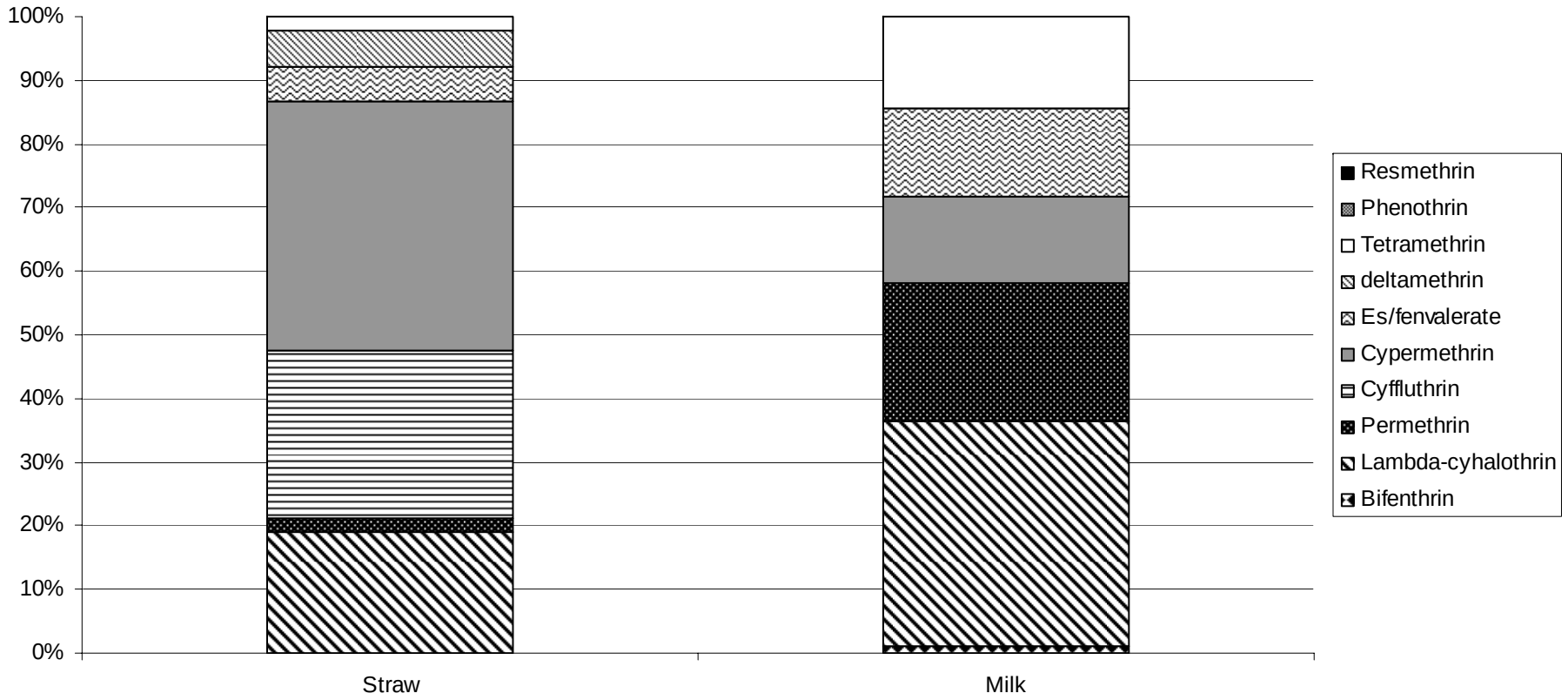
Table 3. Estimated daily intake (EDI) of pyrethroids (expressed as μg (kg bw) $^{-1}$ day^{-1}) calculated from the breastmilk concentrations of these compounds in Manhiça (Mozambique).

	Bifenthrin	λ-Cyhalothrin	Permethrin	Cyfluthrin	Cypermethrin	Es/Fenvalerate	Tetramethrin	∑PYR
Mean	0.05	1.05	0.61	0.61	0.42	0.42	0.62	3.3
Median	0.03	0.85	0.42	0.46	0.03	0.33	0.54	2.8
Max	0.28	3.4	1.75	1.7	0.12	1.5	1.8	9.0
Min	0.01	0.13	0.08	0.09	0.00	0.07	0.05	0.67

Table 4. Median values of pyrethrois concentration in milk samples expressed as mg/kg compared to MRL values reported by WHO FAO 2005.

	Median concentrations in milk samples (mg/kg)	MRL values (mg/kg) FAO WHO 2005.
Cyfluthrin	0.0019	0.04
Deltamethrin	nd	0.05
Cypermethrin	0.0009	0.05
Fenvalerate	0.0128	0.1
Permethrin	0.0059	0.1

Figure 1.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90%
80%
70%
60%
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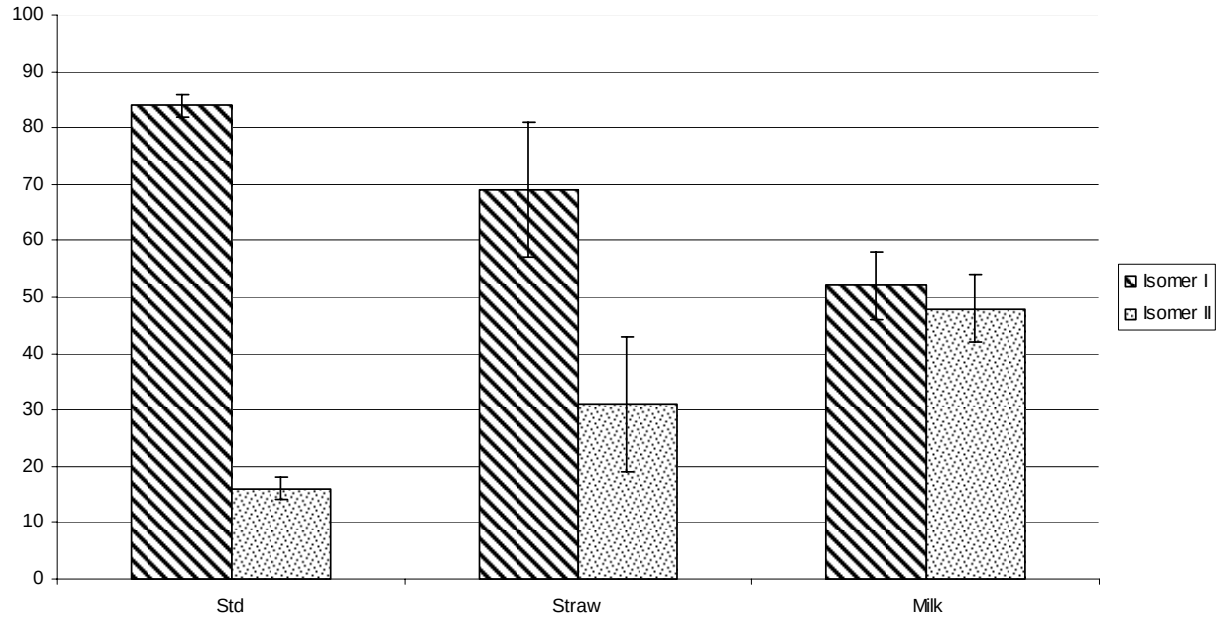
Straw
Milk
Resmethrin
Phenothrin
Tetramethrin
deltamethrin
Es/fenvalerate
Cypermethrin
Cyffluthrin
Permethrin
Lambda-cyhalothrin
Bifenthrin

Figure 2.

a)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80
70
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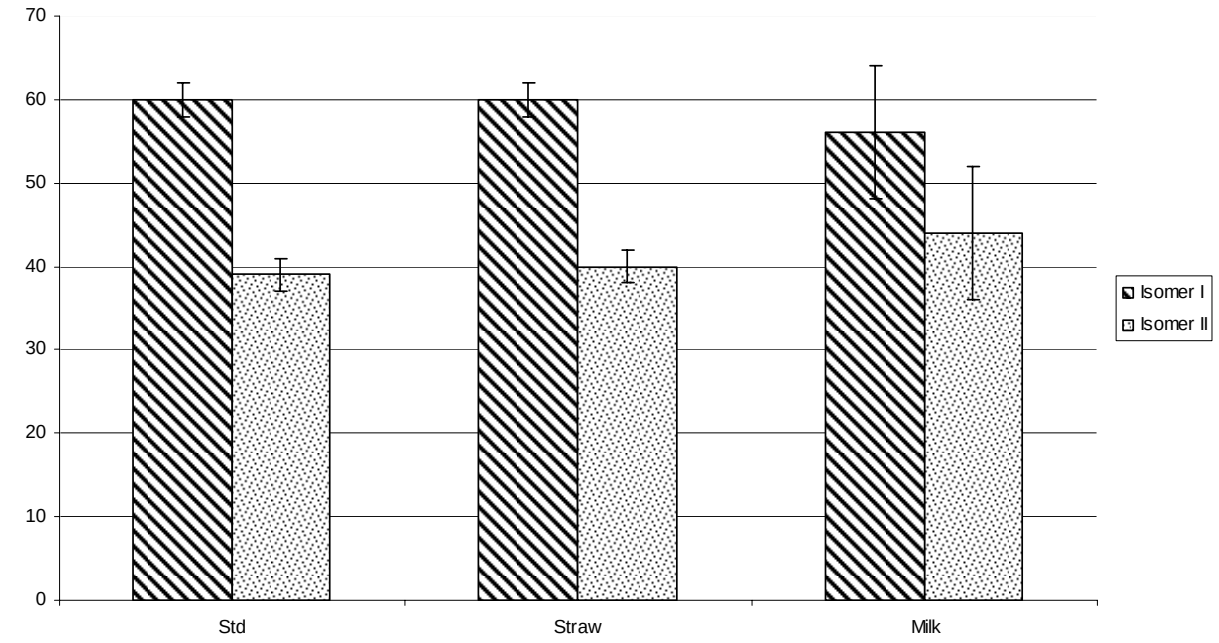
40
30
20
10
0
Std
Straw
Milk
Isomer I
Isomer II

b)


70
60
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10
0
Std
Straw
Milk
Isomer I
Isomer II